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Comparative mapping of bovine chromosome 27 with human chromosome 8 near a dairy form QTL in cattle

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Abstract. In the absence of a complete and annotated bovine genome sequence, detailed human-bovine comparative maps are one of the most effective tools for identification of positional candidate genes contributing to quantitative trait loci (QTL) in cattle. In the present study, eight genes from human chromosome 8 were selected for mapping in cattle to improve breakpoint resolution and confirm gene order on the comparative map near the 40 cM region of the BTA27 linkage map where a QTL affecting dairy form had previously been identified. The resulting map identified *ADRB3* as a positional candidate gene for the QTL contributing to the dairy form trait based on its estimated position between 40 and 45 cM on the linkage map. It is also a functional candidate gene due to its role in fat metabolism, and polymorphisms in the *ADRB3* gene associated with obesity and metabolic disease in humans, as well as, carcass fat in sheep. Further studies are underway to investigate the existence of polymorphisms in the bovine *ADRB3* gene and their association with traits related to fat deposition in cattle.

Body condition may contribute to a dairy cow's susceptibility to a wide variety of diseases, fertility and health problems (Hansen et al., 2002; Lassen et al., 2003), as well as reproductive disorders (Rogers et al., 1999; Starbuck et al., 2004). The genetic correlation between body condition score and dairy form is –0.73 (Dechow et al., 2003). Quantitative trait loci (QTL) associated with dairy form were identified by our laboratory within two regions of bovine chromosome 27 (BTA27) in two Holstein families (Ashwell et al., 2001; Van Tassell et al., 2004). Interval analysis indicated a likely QTL position at 40 cM (95% confidence interval 27–52 cM) containing possibly one or two QTL and the second QTL located near the telomere. Thus the identification of genes contributing to variation in this trait could enhance our ability to modulate body condition and improve animal health and fertility through genetic selection for the most desirable alleles.

Due to the lack of information on the genes present in the regions of BTA27, we must rely on human-bovine comparative chromosome maps to identify positional candidate genes contributing to these QTL. Previous studies indicated that human chromosome 8 (HSA8) or HSA4 may contain regions of conserved synteny near the dairy form QTL located at ~40 cM on the BTA27 linkage map (Sonstegard et al., 2000; Connor et al., 2004). The purpose of the present study was to map genes positioned on human chromosomes which putatively shared con-

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Table 1. Summary of primer sequences, product sizes, annealing temperatures and GenBank accession numbers of source sequences used for PCR amplification of targeted bovine genomic regions

Gene/BAC clone	Primer sequence	Product length (bp)	Annealing temperature (°C)	Accession no./ TIGR TC no.[a]	Putative location
Gene: *ADRB3*	5'-GGAGACCCTTTCCCTCATTC-3' 5'-CAGGAGCGGAAGATAGAACG-3'	700	58.5	NM_174232	exon 1
BRF2	5'-TGCTAACTGGCCTGACACAG-3' 5'-ACCAAGGGATGGGACTCTCT-3'	157	56.0	TC243503	exon 4
CLU	5'-GACGCCCTGAATGACACC-3' 5'-GATCCGCTTCTGCACACC-3'	152	62.0	NM_173902	exon 4
EIF4EBP1	5'-CTAGCCCTACAGGCGATGAG-3' 5'-CAAGATGGTCGCTTGCATAA-3'	~3000	56.0	TC205737	intron 2
FUT10	5'-CCACCTCCATCCGTTCTTTA-3' 5'-GGCTTTGGTCCTGTGATGAT-3'	193	56.0	NM_182987	exon 2
LEPROTL1	5'-TGGCCTCCCTATCGTATTTG-3' 5'-GGCCGAGAAATGACAAGAAG-3'	~1300	58.5	TC247955	intron 3
MFHAS1	5'-TTCTACGGCTGTGGGATTTC-3' 5'-CCTCCCTGCCTCATCTACAG-3'	173	58.5	TC175989	exon 4
MSRA	5'-CACCAGCAGTACCTGAGCAA-3' 5'-CAGCTTGGTTCCCTGAAGTC-3'	400	58.5	NM_174114	exon 11
BAC clone: 303C7	5'-TTTTGTCTGCACTCCCTGTG-3' 5'-GCAGGACTTAAGTGCCCAAA-3'	538	55.0	CC477059	
393M23	5'-GTCTAGCGGGGTCACAAAGA-3' 5'-GGCTGAGCACTGTGTTTGAA-3'	551	55.0	CC592294	
435D11	5'-AGCCACTCGACGTGGTTTAC-3' 5'-CATTTTCAGTGGTGGCCTTT-3'	181	55.0	CC550612	
443K13	5'-AGGGATATGCATGAGGTTGG-3' 5'-CCCTGGTCTCTCTCACACCT-3'	236	55.0	CC568501	

[a] GenBank accession number or TIGR *Bos taurus* Gene Index tentative consensus sequence (TC) number (http://www.tigr.org/).

served synteny with BTA27 and improve the human-bovine comparative map, particularly near the 40 cM region of the BTA27 linkage map, to identify positional candidate genes regulating dairy form.

Materials and methods

Gene selection for mapping

Six genes *(CLU*, *LEPROTL1*, *FUT10*, *ADRB3*, *BRF2* and *EIF4EBP1)* from the 27–39 Mb region of HSA8 and two genes *(MFHAS1* and *MSRA)* from the ~ 5–10 Mb region of HSA8 were selected for mapping on the bovine genome. These genes were selected based on predicted conservation of synteny between this region of HSA8 and BTA27 and available bovine nucleotide sequence information. In addition, four CHORI-240 BAC clones (303C7, 393M23, 443K13 and 435D11) sharing high sequence similarity to HSA8 sequence in the 33–38 Mb region were selected for RH mapping.

Overgo oligo hybridization of CHORI-240 bovine BAC library filter sets (BACPAC Resources, Oakland, CA) was performed according to the methods of John McPherson (http://www.tree.caltech.edu/protocols/overgo.html) using the oligos ovBMS689F (5′-CCCTCTTCAGCTTGCCTCCCTTTC-3′) and ovBMS689R (5′-GAGAAGTAGGATCAGGGAAAGGGA-3′) and ovCSSM036F (5′-TCAACCACACGTCTCTGTCTTTGG-3′) and ovCSSM036R (5′-GGCTTCCAAAGATCGTCCAAAGAC-3′). These overgo probes were designed to hybridize to unique regions flanking bovine microsatellite markers BMS689 (accession no. G18941) and CSSM036 (accession no. U03827), respectively, near our previously identified QTL peak for dairy form (Van Tassell et al., 2004). The BAC contigs containing positive clones identified by the hybridization were identified using iCE v3.4 (http://ice.bcgsc.ca/). BAC end sequences available in the NCBI GenBank database from clones comprising the contig were compared to the Human Genome Draft sequence (April 2003 assembly) using BLAT analysis (http://genome.ucsc.edu/cgi-bin/hgBlat). High scoring alignments (score >250) were identified in the 33–38 Mb region of HSA8; thus genes from this region were targeted for RH mapping in cattle.

Primer design

Table 1 summarizes the primer sequences, annealing temperatures and sources of bovine sequence information used for PCR amplification of each gene target. Primers were designed using Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Amplification of the targeted region was confirmed by agarose gel purification of PCR products (QIAquick Gel Extraction Kit, Qiagen Inc., Valencia, CA) and direct sequencing using a CEQ8000 automated DNA sequencer with Quickstart chemistry (Beckman Coulter, Fullerton, CA). Nucleotide sequences generated for *EIF4EBP1* and *LEPROTL1* were submitted to the GenBank dbSTS database and assigned accession nos. BV209032-BV209035.

RH mapping

Physical mapping of each locus was performed using the Roslin-Cambridge 3000-rad bovine/hamster radiation hybrid panel (Invitrogen Corp., Carlsbad, CA) as previously described (Connor et al., 2004). Amplification by PCR was performed in a 12-µl reaction volume using 50 ng of bovine genomic DNA as template, 0.4 µM of each primer and Platinum PCR Supermix (Invitrogen Corp.). RH maps were constructed using the Carthagene package (Schiex and Gaspin, 1997) as described by Williams et al. (2002).

Results and discussion

Results of RH mapping and two-point analyses are presented in Table 2. Figure 1 illustrates relative marker order on BTA27 based on microsatellite markers included in the previous genetic map (indicated as USDA 97), and physical map (indicated as Williams 2002), with markers typed on the 3000-rad RH panel in the present study (indicated as RH 2004). The addition of our markers described here did not change the number of LOD 6 linkage groups compared to the Williams 2002 map, although marker order in the telomeric linkage

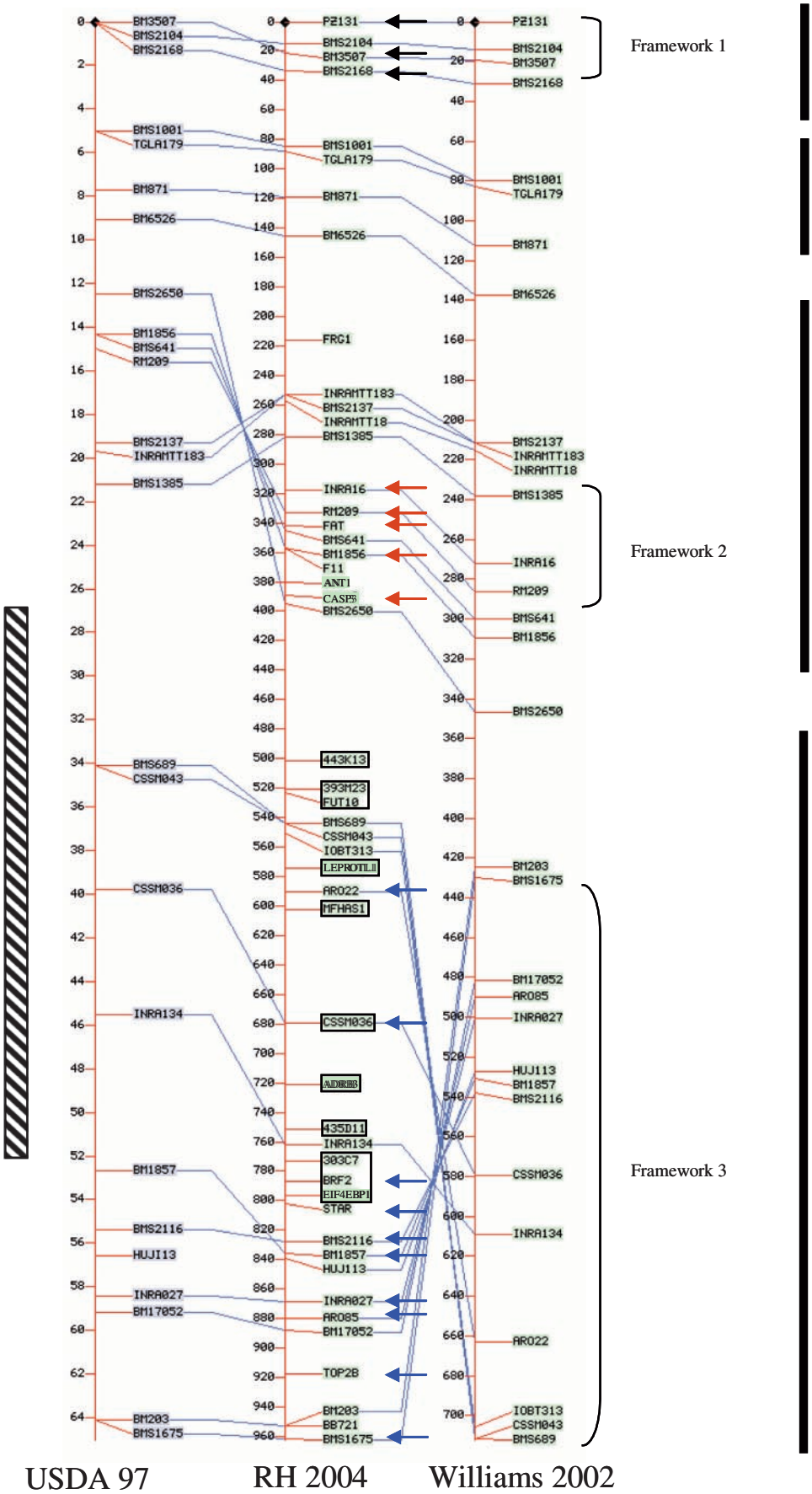

Fig. 1. Genetic and physical maps of chromosome 27. USDA 97 = linkage map from Kappes et al. (1997), units in cM; RH 2004 = physical map based on analysis of markers from the present work, units in cR; Williams 2002 = physical map from Williams et al. (2002), units in cR. Heavy vertical lines to right indicate LOD 6 linkage groups. Arrows indicate framework markers within local frameworks shown with brackets. Hatched bar (left side) indicates 95 % confidence interval of the dairy form QTL. QTL peak is at approximately 40 cM. Boxes surrounding marker names on RH 2004 map indicate loci positioned in the present study.

Table 2. Details of physical mapping of bovine gene- and BAC clone-associated sequence tagged sites

Locus/clone[a]	BTA	Closest locus	2-point LOD	Distance (cR)	HSA ($\times 10^6$ bp)[b]
ADRB3	27	INRA134	11.72	0.30	8(37.94)
BRF2	27	INRA134	20.00	0.08	8(37.82)
CLU	8	IDVGA11	5.35	0.66	8(27.52)
EIF4EBP1	27	*STAR*	20.74	0.05	8(38.02)
FUT10	27	BMS689	13.21	0.19	8(33.39)
LEPROTL1	27	ARO22	15.92	0.16	8(30.02)
MFHAS1	27	ARO22	16.57	0.11	8(8.73)
MSRA	8	*KIAA1712*	11.23	0.30	8(10.14)
303C7	27	INRA134	18.76	0.10	8(38.16)
393M23	27	IOBT313	13.56	0.20	8(33.99)
435D11	27	INRA134	19.64	0.10	8(37.32)
443K13	27	IOBT313	9.88	0.36	8(35.14)

[a] Clones are derived from the bovine BAC CHORI-240 library (http://www.chori.org/bacpac/bovine240.htm). Clone names indicate library plate, row and column locations.
[b] Human Genome Working Draft (May 2004 assembly; http://genome.ucsc.edu/). Number in parentheses is approximate number of bases from the p arm telomere of the chromosome.

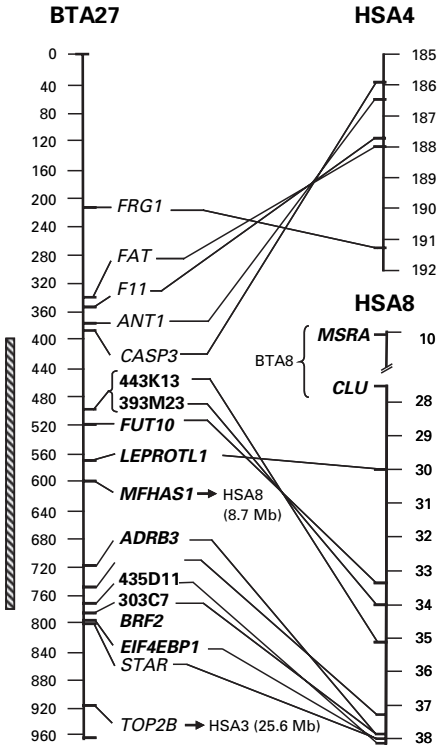

Fig. 2. Comparative alignment of BTA27 and HSA4 and 8 near previously identified QTL for dairy form on BTA27 (hatched bar to left indicates 95 % confidence interval). Estimated positions for the bovine map are in cR. Distances on HSA8 are in Mb from the p arm telomere based on Human Genome Working Draft (May 2004 assembly; http://genome.ucsc.edu/). Locus symbols indicated in bold were positioned in the present study. Previous assignments are from Connor et al. (2004) and were repositioned using Carthagene software.

group was inverted. This inversion is consistent with marker order on the USDA 97 map and covers the majority of the QTL interval. In contrast, the orientation of the next linkage group towards the center of the chromosome (from markers *FRG1* to BMS2650) is consistent between the two physical maps but inverted relative to the linkage map. Placement of additional markers on the physical and genetic maps will aid in their future integration.

Two gene loci selected from HSA8 for mapping, *MSRA* (at 10.1 Mb) and *MFHAS1* (at 8.7 Mb), were positioned on BTA8 and BTA27, respectively (Table 2). Mapping of *MSRA* is consistent with reports of Everts-van der Wind et al. (2004) and aids in the integration of the Roslin 3000-rad RH map and the ILTX-2004 second-generation 5000-rad RH map. Relative to the human genome sequence, the nearest gene marker to *MFHAS1* positioned on both the human and bovine maps is *DEFB1* (at 6.7 Mb on HSA8), which maps near the center of BTA27. Thus mapping of *MFHAS1* to the bovine genome extends the known region of conserved synteny on HSA8 with BTA27 by approximately 2.0 Mb, and narrows the location of the chromosomal breakpoint in the human-bovine comparative map to a 1.4-Mb region of HSA8, between *MSRA* and *MFHAS1*.

Mapping of *CLU* to BTA8 close to marker IDVGA11 (Table 2) is in agreement with previous reports (Band et al., 2000; Sonstegard et al., 2000; Goldammer et al., 2004) and corresponds to approximately 9 cM on the bovine linkage map. This places *CLU* in close proximity to *MSRA* in the bovine genome since *MSRA* was close to *KIAA1712*, a gene previously mapped to the same region of BTA8 (Connor et al., 2004). Our findings are consistent with the map of BTA8 from Everts-van der Wind et al. (2004; http://cagst.animal.uiuc.edu/RHmap2004/) and indicate the small region of BTA8 between *CLU* and *MSRA* corresponds to two separate regions of HSA8. Of interest, *KIAA1712* is located on HSA4 at approximately 175.8 Mb. Thus the region of BTA8 between *MSRA* and *KIAA1712* contains a breakpoint in the human-bovine comparative map corresponding to regions of HSA8 (at ~10.1 Mb) and HSA4 (at ~175.8 Mb), respectively.

The remaining loci, *FUT10*, *LEPROTL1*, *MFHAS1*, *ADRB3*, *BRF2*, *EIF4EBP1* and the STS markers 303C7, 393M23, 443K13 and 435D11 were mapped to BTA27 near the QTL region (Fig. 2). Mapping of these loci improves the marker density on the human-bovine comparative map primarily in the 30–38 Mb region of HSA8. Mapping of *EIF4EBP1* to BTA27 is consistent with findings of Goldammer et al. (2004), although placement of *EIF4EPB1* relative to microsatellite marker CSSM036 is inverted compared to their map.

Based on multipoint analysis results, several rearrangements in gene order appear within small chromosomal segments of HSA8 compared to BTA27 within our QTL region (Fig. 2). Most significantly, *MFHAS1* is positioned between *LEPROTL1* and *ADRB3* in the bovine genome, but near *MSRA* in the 8.7 Mb region of HSA8. This result suggests chro-

mosomal regions on BTA27 between marker 443K13 and *STAR* correspond to two separate regions of HSA8, one in the 8.7 Mb region and one in the 30–40 Mb region. Overall, our mapping results suggest genes positioned between *CASP3* and *BRF2* are positional candidate genes contributing to the dairy form QTL located in the center of BTA27 (Fig. 2).

Dairy form is a subjective measure encompassing multiple characteristics primarily relating to the degree of fat deposition around the neck, shoulders, hips and topline of a dairy cow. A cow exhibiting a high dairy form score indicates the ability to mobilize energy into milk production rather than body reserves, hence the desirability of the trait. However, extreme dairy form can also be related to an increased susceptibility to metabolic diseases such as ketosis and reduced fertility (Rogers et al., 1999). Based on the factors contributing to the trait, the most obvious functional candidate within the chromosomal interval containing the QTL on BTA27 is *ADRB3* (beta-3-adrenergic receptor); a gene expressed primarily in adipose tissue that regulates energy metabolism.

In sheep, associations between allelic variation at the *ADRB3* locus and measures of carcass fat have been demonstrated (Forrest et al., 2003) and numerous studies examining a Trp64-to-Arg mutation in the human *ADRB3* gene have found relationships between the mutation and metabolic disease and obesity (NCBI OMIM No. 109691). In cattle, there have been no reports of variation in the amino acid sequence of ADRB3 protein. Lastly, Revelli et al. (1997) developed *ADRB3* knockout mice and found them to be more susceptible to obesity than wild-type mice. These observations suggest that *ADRB3* may mediate body fat deposition in dairy cattle and a variety of phenotypic traits including dairy form.

We performed a preliminary sequence analysis to identify polymorphisms near the *ADRB3* gene in the Holstein family segregating for the dairy form QTL. A single indel within the putative promoter of the *ADRB3* gene was identified for which the segregating sire was heterozygous. However, some sires that do not appear to be segregating for the QTL also exhibited the same heterozygous genotype for the indel, suggesting that this polymorphism is unlikely to be associated with the dairy form trait.

In conclusion, eight genes from the 0–40 Mb region of HSA8 and four bovine CHORI-240 BAC end sequence-associated STSs sharing high sequence similarity with the 33–38 Mb region of HSA8 were selected for radiation hybrid mapping to the cattle genome. This information was used to improve the comparative map near the 40 cM region of the BTA27 linkage map where a QTL for dairy form had been previously identified in a Holstein cattle population. The gene *ADRB3*, located at approximately 40–45 cM on BTA27, was identified as a positional candidate gene contributing to the QTL based on the role of this gene in fat metabolism and its association with metabolic diseases and fat deposition in sheep, mice and humans. Additional work currently is underway to determine whether polymorphisms in the bovine *ADRB3* gene exist in dairy families segregating for the dairy form QTL and to study the associations between *ADRB3* and health and production traits such as dairy form, metabolic disease, and fat deposition in cattle. Further analysis and comparative mapping of HSA8 near the *MFHAS1* locus is needed to determine whether additional functional candidate genes can be identified within the dairy form QTL interval on BTA27.

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